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Abstract

Title of Thesis: Melanopsin Polymorphisms in Seasonal Affective Disorder

Kathryn Ariel Roecklein, Master of Science, 2005

Thesis directed by: Kelly J. Rohan, Ph.D.

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Seasonal affective disorder (SAD) is characterized by winter depressive episodes and springtime remission. SAD may result from a genetically-mediated abnormal response to low light availability during winter. One candidate gene for SAD is melanopsin, a non-visual, circadian photopigment. The present study determined the frequency of a genetic polymorphism in melanopsin (P10L) in individuals with SAD (n = 36) compared to two groups: gender-matched controls with no history of depression and minimal seasonality (n = 22) and a larger comparison group of samples obtained from NIH that have been delinked from identifying information (n = 84). The proportion of SAD participants with P10L (28%) did not differ significantly from the comparison group (15%) or nondepressed controls (18%). A post-hoc power analysis revealed that a sample of 200 participants would be required in future studies. If a sufficiently sized sample including gender- and ethnicity-matched controls becomes available, then the study should be repeated.

MELANOPSIN POLYMORPHISMS IN SEASONAL AFFECTIVE DISORDER

by

Kathryn Ariel Roecklein

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Introduction

Winter seasonal affective disorder (SAD) was first characterized by Rosenthal et al. (1984) as a syndrome involving recurrent Major Depressive Episodes in fall and winter that remit during spring and summer. Etiological hypotheses and treatment outcome research suggest that SAD may result from reduced natural light availability in the winter. Individuals with SAD have abnormal responses to light, including circadian rhythm disturbances, abnormal retinal sensitivity, and an antidepressant response to light therapy. We have hypothesized that SAD etiology may involve a genetically-mediated disturbance in the non-visual light input pathway from the retina to the suprachiasmatic nuclei (SCN), the location of the central circadian clock in mammals. The first step in the non-visual light input pathway is photon capture by photoreceptors in the retina, including melanopsin, a non-visual photoreceptor found in retinal ganglion cells (Panda et al., 2003; Panda et al., 2002; Ruby et al., 2002). Findings regarding melanopsin's role in non-visual photoreception in mammals are presented as background in this Master's thesis write-up because these findings may be analogous to the role of melanopsin in humans. Studies on markers of photoperiodic responses in rodents missing the melanopsin gene and humans with SAD are presented to support a possible role for melanopsin in SAD. Also, recent studies with human twins raised apart and family studies are presented to support the hypothesis that SAD may have a partly genetic basis (Sher, Goldman, Ozaki, & Rosenthal, 1999). The research described in this write-up was an attempt to determine whether changes in the genetic sequence of melanopsin (i.e., polymorphisms) may be associated with SAD.

Melanopsin and Non-Visual Photoreception

The eye has two basic tasks: photoreception for vision and photoreception for non-visual tasks, such as circadian photoreception (Provencio, Rollag, & Castrucci, 2002). Circadian rhythms are rhythmically-expressed biological processes that recur about every 24 hours and are synchronized with photoperiod (i.e., daylength) onset through retinal input (i.e., photoreception). Photoreceptors in the retina contain the light-sensitive, protein-based photopigments. The retina collects environmental light in the form of photons, converts them to neural signals, and transmits these signals to brain areas including the circadian clock (Kandel, Schwartz, & Jessell, 2000). One member of the opsin family (i.e., a class of proteins that are light sensitive), the photopigment melanopsin, was originally found in amphibian cells that darken in response to light (Daniolos, Lerner, & Lerner, 1990). In humans, melanopsin is expressed only in the inner retina, not in the rods and cones which are responsible for visual photoreception, but in a subset of intrinsically photosensitive retinal ganglion cells (ipRGCs; Gooley, Lu, Chou, Scammell, & Saper, 2001; Hannibal, Hindersson, Knudsen, Georg, & Fahrenkrug, 2002; Provencio et al., 2002). These particular ipRGCs project to brain areas involved in non-visual responses to light, including visuomotor responses to light and circadian rhythms (Horowitz, Blanchard, & Morin, 2004; Kandel et al., 2000). Melanopsin is likely to be the molecule conferring photosensitivity to ipRGCs (Provencio, 2004).

The classical visual photoreceptors, rods and cones, as well as the non-visual photoreceptors, such as those containing melanopsin, are involved in light input to the circadian clock and other non-visual brain areas (Provencio, 2004). Non-visual photoreceptive tasks mediated by the retina in mammals include: (1) behavioral

rhythmicity including circadian photoreception, (2) the pupillary light response (Van Gelder, 2001), (3) pineal melatonin synthesis, as well as functions potentially mediated by other retinorecipient areas of the brain including sleep and mood regulation. Each of these tasks is described below, highlighting melanopsin's potential role. The molecular bases of these non-visual tasks have not been completely described to date. The recent identification of ipRGCs and characterization of melanopsin's role in rodent circadian photoreception and the pupillary light response led members of our laboratory to speculate that melanopsin may have an analogous role in humans, and further, that melanopsin variations may be involved in SAD.

Circadian Rhythms and Melanopsin

Light input to the circadian clock allows the body to anticipate dawn and coordinate physiological functions with environmental conditions. Photoentrainment is the process of synchronizing the body's internal circadian rhythms, such as sleep or activity onset, with the environment's light-dark cycle. Photoentrainment is adaptive given the necessity for diurnal and nocturnal organisms to be awake and sleeping at specific times of day and to anticipate dawn and dusk to prepare for periods of activity. Because photoperiod changes daily and deviates by a matter of hours from season to season, entrainment must be adjusted on a daily basis through non-visual light input (Provencio, 2004).

Melanopsin is involved in non-visual responses to light in rodents. Light can induce circadian phase-shifts in melatonin levels in individuals without normal color vision (Ruberg et al., 1996), suggesting that the cones are not the only photoreceptors involved in non-visual responses to light. Research with mice that are missing the rod

and cone cells responsible for vision (retinally degenerate mice; *rd/rd*) confirms that in these mice, circadian photoreception is at least partly independent of normal visual cells and projections (Menaker, 2003; Panda et al., 2002; Provencio, Wong, Lederman, Argamaso, & Foster, 1994; Ruby et al., 2002; Yoshimura & Ebihara, 1996). Transgenic mice lacking both copies of the melanopsin gene ($Opn4^{-/-}$) have been created recently, allowing researchers to investigate the role of melanopsin in non-visual responses to light including circadian photoentrainment, the pupillary light reflex, and acute suppression of nocturnal melatonin release (Lucas et al., 2003). Despite similar nomenclature, melatonin and melanopsin have distinct functions and localizations in the body. Melatonin is a hormone synthesized in the pituitary that is released at night and may have a sleep-inducing effect in humans (Kandel et al., 2000), whereas melanopsin, the focus of this study, is a non-visual photopigment found only in the retina in humans (Provencio et al., 2000). In addition, mice lacking melanopsin as well as rods and cones ($Opn4^{-/-}$; *rd/rd*) have been created, allowing comparison of *rd/rd* with $Opn4^{-/-}$; *rd/rd* to isolate the impact of melanopsin (Girkin, 2003). The non-visual system of rodents may be analogous to that of humans, and human circadian and sleep disorders may be traceable to the molecular components of the non-visual system. SAD may be the result of abnormal light input, suggesting that investigating the role of melanopsin in SAD may identify a molecular mechanism for SAD.

Phase-Shifting to Light: Role of Melanopsin

The neural connection between the retina and the circadian clock allows the clock to identify time of day relative to the time of onset of internal circadian rhythms and to entrain or synchronize internal events with environmental events. Light can cause a shift

in the reference point of an internal rhythm, if timed appropriately, creating either a phase-advance or phase-delay. As one example of a circadian rhythm that can be phase-shifted by light, melatonin is a rhythmically-released hormone with sleep-inducing properties in humans and sleep- and breeding-related effects in rodents (Kandel et al., 2000). Melatonin levels follow a circadian rhythm with a nocturnal maximum and a diurnal minimum. Rhythms such as the onset of the nocturnal release of melatonin are closely linked to the central clock and are used as markers of circadian phase in rodents and humans. Research with Opn4$^{-/-}$ mice found that the degree of phase-shift in response to light input was significantly attenuated among melanopsin knockout mice (Panda et al., 2002; Ruby et al., 2002). These data suggest that, at least in mice, melanopsin is critical to circadian photoentrainment (Panda et al., 2003), but is not the only photopigment involved (Menaker, 2003). Pulses of bright light acutely suppress the nocturnal release of melatonin. Arylalkylamine-N-acetyltransferase (AA-NAT) transcript levels reflect synthesis of melatonin and are inhibited by light. In mice missing the melanopsin gene and rods and cones (*Opn4*$^{-/-}$; *rd/rd*), AA-NAT levels were not inhibited by light as they are in *rd/rd* mice that only have ipRGCs (Girkin, 2003). Failure of AA-NAT suppression in *Opn4*$^{-/-}$; *rd/rd* mice suggests that rods, cones, and ipRGCs are involved in this response. Therefore, melanopsin appears to play a critical role in melatonin suppression in response to light.

Pupillary Light Reflex: Role of Melanopsin

Bright light causes the pupil to constrict, enhancing depth of focus, reducing light scatter, and facilitating dark adaptation (Girkin, 2003). This reflex is mediated by RGCs projecting to the olivary pretectal nucleus (OPN), and then bilaterally to the Edinger-

Westphal nuclei, causing the oculomotor cranial nerves to contract the pupillary sphincter muscles (Van Gelder, 2001). Mice without rods and cones retain this pupillary light response, although latency is increased and magnitude of response is diminished (Girkin, 2003). The pupillary light response is used to segregate groups of blind individuals into those who do or do not retain afferent retinal projections to the brain (Lucas et al., 2003).

Lucas et al. (2003) reported that melanopsin knock-out mice (*Opn4*$^{-/-}$) had normal pupillary light responses at low irradiances, but had an incomplete reflex at higher irradiances. These data suggest that the visual rod and cone system works in combination with melanopsin to mediate the pupillary light reflex (Menaker, 2003). Data from *Opn4*$^{-/-}$ and *rd/rd* mice show the same pattern of results for phase-shifting, melatonin suppression and pupillary response. Therefore, melanopsin plays an important role in these responses, as do other photoreceptors (Menaker, 2003). A partially diminished response to light in the non-visual system of humans because of melanopsin variations could explain the observed abnormal response to low light levels in SAD described below.

Abnormal Response to Low Light Levels in SAD

Several lines of evidence suggest that SAD may be the result of an abnormal response to the relatively low levels of environmental light during the winter. The relationship between SAD prevalence and photoperiod or time of year implicates the circadian system, of which melanopsin is one component. Similarities between symptoms of SAD in humans and seasonal behavior in other mammals suggests humans may respond to change of season in a manner analogous to that of mammals that use the circadian clock to monitor time of year. The efficacy of bright light therapy to treat SAD suggests that non-visual responses to light may play a role in the etiology and treatment

of SAD. Polymorphisms in the gene for melanopsin could lead to an abnormal response to non-visual light input, which may be one explanation for the abnormal response to light seen in SAD. The nature of the abnormal responses to light observed in SAD are described below.

Latitude and Photoperiod

Photoperiod is determined by two factors: day of year and latitude. Early SAD researchers postulated that SAD would be more common at extreme Northern or Southern latitudes because of the progressively shorter winter photoperiods as distance from the equator increases. Indeed, initial epidemiological surveys at varying latitudes in the U.S. found evidence for increasing rates of SAD with more Northern latitudes, ranging from 9.7% in New Hampshire to 1.4% in Florida (Mersch, Middendorp, Bouhuys, Beersma, & van den Hoofdakker, 1999). Subsequent studies found that latitude accounts for a smaller percentage of variance in SAD prevalence rates outside of North America (Molin, Mellerup, Bolwig, Scheike, & Dam, 1996). Individuals with a deficiency in responding to non-visual light input, perhaps mediated by low levels of functional ipRGCs, could be more vulnerable to SAD as photoperiod decreases with increasing latitude. Polymorphisms in the gene for melanopsin could lead to an abnormal sensitivity to non-visual light input that may make individuals living at extreme latitudes more vulnerable to SAD.

SAD Onset and Environmental Light Levels

Studies have found a correlation between some climatic variables and SAD. Environmental light-related paratmeters that vary with season include photoperiod and the intensity of environmental light (minutes of sunshine, cloud cover, global radiation).

Depression severity in SAD as measured by the Beck Depression Inventory (BDI; Beck, Steer, & Brown, 1996; Molin et al., 1996), correlates negatively with photoperiod and overall intensity of available light as measured by global radiation and total minutes of sunshine and positively with cloud cover. Other research has correlated photoperiod (as measured by daily minutes of sunlight) and intensity of light (as measured by global radiation) with SAD epsidode onset. Only photoperiod emerged as a significant predictor of SAD episode onset (Young, Meaden, Fogg, Cherin, & Eastman, 1997). These data suggest that individuals with SAD have an abnormal response to shortening photoperiod in winter, a response that could be the result of having too few functional photoreceptors. Insufficient numbers of non-visual photoreceptors could mean that low light levels in winter fall below the threshold required for proper neural signaling in the non-visual light input pathway.

Circannual Rhythms and Photoperiod

In some rodents, seasonal (circannual) rhythms in rest and activity are observed, including winter hibernation (Sharma, 2003). Whereas circadian rhythms are ubiquitous in most animals, it is not clear whether humans have circannual rhythms (Wehr et al., 2001). The seasonal presentation of depression symptoms in SAD suggests that humans with SAD may have possible circannual rhythms in mood and behavior among individuals with SAD. Alternatively, SAD patients may experience biological changes to circadian signals of season such as changing photoperiod (Wehr, 2001). SAD is characterized by both circannual recurrence, and abnormalities in circadian rhythms for sleep, appetite, mood, energy or arousal, melatonin release, and core body temperature (APA, 2000; Teicher et al., 1997).

Some individuals with SAD experience symptoms similar to the behaviors observed in hibernating animals. Major Depression can include the symptoms of depressed mood; loss of interest in activities; changes in appetite, weight, and sleep; psychomotor retardation or agitation; fatigue or loss of energy; feelings of guilt or worthlessness; difficulty concentrating; and recurrent suicidal ideation (APA, 2000). Relative to individuals with nonseasonal depression, individuals with SAD are more likely to experience atypical or reverse-vegetative symptoms such as increased appetite and weight gain, carbohydrate craving, hypersomnia, and anergia (Tam et al., 1997). Carbohydrate craving in SAD is unique in that both a winter seasonal peak and a daily afternoon peak in the craving and eating of starches and sweets are observed (Arbisi, Levine, Nerenberg, & Wolf, 1996). The atypical depression symptoms seen in SAD are similar to seasonal changes in hibernating animals including decreased activity, hypersomnia, increased appetite, weight gain, reduced metabolism, decreased reproductive behavior, and decreased interaction with the environment (Wehr, 1999). The similarity between seasonal presentation of behavioral changes in SAD and seasonal animal behavior suggests a common biological mechanism (Wehr, 2001).

The most predictable environmental parameter associated with the change of season is photoperiod (Provencio, 2004). In animals, changes in photoperiod signal the pineal gland to lengthen or shorten the duration of melatonin release at night which, in turn, produces changes in the reproductive drive, activity level, sleep, feeding, weight, and metabolism (Wehr, 1995). As mentioned above, *rd/rd;Opn4*$^{-/-}$ mice do not suppress melatonin synthesis in response to light as do wild type mice (Panda et al., 2003). It is possible that humans with a genetic variation in melanopsin also may fail to respond to

light with acute melatonin suppression and experience a lengthened duration of melatonin synthesis when light levels are lower in the winter. A lengthened duration of nocturnal melatonin release in winter could be one mechanism for the seasonal presentation of SAD symptoms, analogous to the mechanism for seasonal behaviors in rodents. Variations in the gene for melanopsin may make certain individuals less sensitive to light's suppression of melatonin release, leading to a lengthened duration of release.

In fact, a study of the duration of nocturnal melatonin release found evidence for seasonal rhythms in individuals with SAD, but results varied on the basis of how the duration of nocturnal melatonin release was measured (i.e., the duration of active nocturnal melatonin release, vs duration in which any melatonin was detectable in plasma). In individuals with SAD, the duration of active nocturnal melatonin release was longer in winter than in summer, but in healthy controls, winter and summer did not differ (Wehr et al., 2001). Using detectable melatonin in plasma, only men with SAD evidenced a longer duration in winter compared to summer, whereas women with SAD and healthy controls did not differ in winter compared to summer (Wehr et al., 2001). A lengthened melatonin release duration in some individuals with SAD suggests that humans have retained the ability to track seasonal change physiologically but that this "circadian signal of change of season" (p. 1108, Wehr et al., 2001) may be masked by modern indoor lighting in the natural environment in individuals without SAD (Graw, Recker, Sand, Krauchi, & Wirz-Justice, 1999). Because men with SAD experienced this seasonal change in melatonin profile, individuals with SAD may respond differently to environmental light cues in the naturalistic environment compared to individuals without SAD (Wehr et al., 2001). It is possible that artificial light levels are of insufficient

intensity or duration to normalize the duration of nocturnal melatonin release in individuals with SAD. As one part of the environmental light input pathway, melanopsin may underlie this type of seasonal change in SAD.

Retinal Subsensitivity in SAD

Individuals with SAD have diminished retinal sensitivity to light (Graw et al., 1999). The retinal sensitivity hypothesis proposes that the retina, as a whole, is less sensitive to environmental light cues in SAD, and that this insensitivity may result from abnormalities in rods, cones, ipRGCs, or other retinal photoreceptors (Hebert, Dumont, & Lachapelle, 2002). Normally, under low light conditions, the retina increases its sensitivity to light in order to maintain proper functioning. This up-regulation of sensitivity may not occur in individuals with SAD (Hebert et al., 2002). Specifically, SAD patients may have a decreased sensitivity to the amount of light they receive year-round. If so, then available photons for retinal absorption in winter may fall below the threshold for normal function of the non-visual light input pathway.

Evidence for retinal subsensitivity in SAD comes from naturalistic studies of light exposure profiles in SAD and non-SAD individuals across the seasons. Women with SAD spend more time outdoors in the summer, but not in the winter, than non-SAD controls (Hebert et al., 2002). This finding implies that individuals with an increased need for light, perhaps those with SAD, may increase their light input in the summer by going outside, but may be unable to do this in the winter because of low temperatures, later dawn, and earlier dusk, resulting in SAD. A greater need for light, resulting from a decreased retinal sensitivity, supports a role for melanopsin in SAD because individuals with fewer functional photoreceptors experience reduced biologic sensitivity to light.

Individuals with fewer functional ipRGCs may also show decrements in retinal sensitivity similar to those identified in SAD (Hebert et al., 2002; Murphy et al., 1993; Ozaki, Rosenthal, Myers, Schwartz, & Oren, 1995; Terman & Terman, 1999).

Circadian Abnormalities in SAD

Further evidence linking melanopsin and SAD comes from observed circadian rhythm abnormalities in SAD. Specifically, circadian abnormalities in mood, sleep, core body temperature, and neuroendocrine secretion have been reported in SAD (Schwartz et al., 2000). Circadian sleep disturbances include hypersomnia, increased sleep latency, and an increase in slow-wave sleep (Avery et al., 1997; Dahl et al., 1993; Khalsa, Jewett, Duffy, & Czeisler, 2000; Shanahan & Czeisler, 1991).

A confound in interpreting the phase of circadian rhythms is that these rhythms are vulnerable to perturbation by activity such as sleep and waking behavior in addition to environmental light information. For example, a delay in sleep onset may be due to either a delay in the central clock, a delay in the sleep homeostat, delayed sensitivity to the sleep homeostat, or masking of sleep onset by late night activity. Therefore, the timing of sleep may not directly reflect the phase of the circadian clock and can be influenced by recent sleep history and activity levels. Some rhythms, including core body temperature and melatonin secretion, are more closely linked to the circadian clock and are relatively less affected by activity rhythms, although care must be taken to minimize the effects of activity and sleep in measuring these rhythms (Dahl et al., 1993).

Some studies have found that rhythms in core body temperature, cortisol profile, and dim light melatonin onset (DLMO) are phase-delayed in some individuals with SAD and can be advanced with light therapy (Avery et al., 1997; Czeisler et al., 1989).

However, one study found no difference in the time of onset of nocturnal melatonin release in individuals with SAD compared to controls (Wehr et al., 2001). Instead, the Wehr et al. (2001) study found that the offset time of melatonin secretion was delayed in winter in patients with SAD compared to summer, but this phase delay was not found in healthy volunteers. Because administering light in the morning can phase-advance circadian rhythms, and because melanopsin is involved in phase-shifting responses to light, melanopsin deficiencies could underlie the circadian abnormalities in SAD.

Antidepressant Effect of Light in SAD

A final piece of evidence suggesting a role for melanopsin in SAD is the demonstrated antidepressant effect of bright light in treating SAD. Bright light therapy is the most common and best available treatment for SAD (Terman et al., 1989). Light therapy may improve SAD because of the importance of light to proper functioning of the circadian clock. Light resets the internal clock even when individuals are isolated from other time cues, demonstrating that light is the primary environmental cue used by the circadian clock to determine time of day (Magnusson & Boivin, 2003). However, it is important to remember that circadian photoentrainment is only one non-visual response to light mediated by melanopsin. Light may be antidepressant in SAD independent of its impact on circadian rhythms. Because light has a variety of effects on the body, researchers have attempted to manipulate several light-related parameters with the aim of alleviating SAD, such as timing, intensity, and wavelength (Lee, Chan, Paterson, Janzen, & Blashko, 1997; Oren et al., 1991). Research using bright light therapy to shorten the nocturnal duration of melatonin release, to simulate a summer-like photoperiod, to phase-advance circadian rhythms and to increase the number of photons delivered to the retina

is described below. The details of how light therapy has been used in SAD and the parameters that have been effective suggest a role for melanopsin in mediating the antidepressant effect of light on SAD.

Light therapy is an effective treatment for SAD.

A recent meta-analysis of light therapy for SAD found an average effect size of 0.84 (95% CI = 0.6-1.08), which is equivalent to effect sizes found in antidepressant medication trials for depression, and an average odds ratio of 2.9 (95% CI = 1.6-5.4) for remission, indicating that light therapy is about three times more likely than credible placebos to produce full remission (Gaynes et al., 2003). Across studies, 53% of individuals with SAD overall and 43% of moderate to severe cases remit with a supervised trial of morning light therapy (Terman et al., 1989). However, compliance with the light therapy prescription diminishes after the initial protocol (41% compliance in subsequent year; Schwartz, Brown, Wehr, & Rosenthal, 1996).

Combined morning and evening light: Skeleton photoperiod to simulate summer.

In many animals and plants[i], seasonal rhythms depend on the measurement of daily photoperiod and light-induced changes in the duration of nocturnal melatonin release (Wehr et al., 1986). Whereas in the natural environment, photoperiod is a constant duration of illumination, a “skeleton” photoperiod can be simulated in the laboratory with brief pulses of light at the beginning and end of the simulated photoperiod with darkness in the interim. To simulate a long photoperiod in animals, brief pulses of light administered before dawn and after dusk are sufficient for entrainment to a long photoperiod. Constant illumination during the typical daylight hours is not required to achieve photoentrainment to a long photoperiod, suggesting that

light in the morning and evening, rather than the entire light period is the critical factor in animals (Wehr et al., 1986). For SAD patients, a skeleton photoperiod is simulated by initiating light therapy before dawn and after dusk rather than during the daylight hours (Wehr et al., 1986). A skeleton light therapy regimen simulating a long summer photoperiod (e.g., light between 7:30 - 10:00 a.m. and 8:00 - 11:00 p.m.) was compared to a regimen that did not extend the photoperiod (e.g., light therapy between 9:00 a.m. and noon, and from 2:00 to 5:00 p.m.). No differences in efficacy were found between the long and short photoperiod regimens, indicating that timing light administration to lengthen photoperiod was not more effective than light therapy during the daylight hours of a winter day (Terman, Terman, Lo, & Cooper, 2001).

Morning light to phase-advance circadian rhythms.

The antidepressant effect of light may be related to light's ability to advance abnormally delayed circadian rhythms in SAD (Lewy, Sack, Singer, & White, 1987). Terman et al.'s (1989) quantitative review of light therapy's efficacy concluded that light therapy administered in the early morning had a higher remission rate (53% remission) relative to light therapy in the evening (38% remission) or midday (32% remission). There is no evidence that light in the evening makes SAD symptoms worse.

Most studies since this meta-analysis have found that morning administration of light is more effective than evening light, leading researchers to recommend that light therapy be scheduled immediately upon awakening (Meesters, Jansen, Beersma, Bouhuys, & van den Hoofdakker, 1995; Wehr et al., 1986; Wirz-Justice et al., 1993). However, other researchers have found that morning light is comparable in efficacy to evening light or to dual morning-evening administration, replicating the findings of the

skeleton photoperiod study (Lee et al., 1997). Lee et al.'s (1997) meta-analysis of 40 light therapy trials found that the morning-plus-evening combination demonstrated greater effect sizes (d = 2.09) than morning alone (d = 1.74), evening alone (d = 1.35), or mid-day (d = 1.27) light administration. Being a meta-analysis of light therapy trials, the Lee et al. (1997) study may have conducted the most statistically powerful test comparing light therapy regimens. Because morning and evening pulses of light are most important for photoentrainment in animals, perhaps in humans with SAD the superior effect of morning and evening light combined is similarly related to photoentrainment and melanopsin containing circadian photoreceptors.

Terman et al. (2001) investigated the antidepressant effect and phase-shift response to morning and evening light in individuals with SAD. Results suggest that there may be a relationship between the magnitude of phase-advance caused by morning light and the degree of antidepressant response (Terman et al., 2001). However, no differences were found for depression scores between groups receiving morning and evening light. Heterogeneity in the findings of treatment studies suggests heterogeneity in the pathogenetic role of light in different individuals with SAD. Melanopsin may be one of a number of molecules involved in the non-visual light system that could underlie differing vulnerabilities in sub-groups of individuals with SAD. Alternatively, different genetic factors within the melanopsin gene may have differing functional consequences that could explain the heterogeneity of findings in light therapy trials. Therefore, investigating the entire sequence of melanopsin in individuals with SAD is important.

Light Therapy and the Photon-Counting Hypothesis

Because not all research supports morning light therapy over evening or dual morning-evening administration, Rosenthal et al. (1993) suggested that light therapy may be important because it increases the overall number of photons reaching the retina. The essence of the photon-count hypothesis is that shorter photoperiods and/or less intense light in the winter result in an insufficient dose of light (i.e., fewer photons) to the retina in SAD-vulnerable individuals (Rosenthal et al., 1993).

Researchers in our laboratory hypothesize that a malfunction in the transmission of light information to the brain is a plausible biological mechanism for the photon-counting hypothesis because it would suggest that lower environmental light levels in the winter could fall below a threshold required to prevent depression (M.D. Rollag, personal communication, October 11, 2004). We further propose that a deficiency in functional melanopsin photopigments could raise the threshold for light input required to maintain normal function of the non-visual light input system, making individuals with a melanopsin variant vulnerable to depression during the winter.

Absorption profile and light therapy wavelength.

An absorption profile provides information regarding the optimal wavelength of light to induce a particular effect and, therefore, can help to identify the photopigment responsible for the effect. The most potent wavelength for mediation of light-induced melatonin suppression is between 446-477 nm (Brainard et al., 2001). Light therapy using short to medium wavelengths (i.e., 420-580 nm such as blue, green, and yellow light) is more beneficial than longer wavelengths (i.e., red light) to individuals with SAD (Lee et al., 1997). The most effective wavelength for inducing circadian responses in

rodents is similar to that absorbed by the opsins, including melanopsin, rather than other types of light-sensitive molecules such as cryptochromes (Provencio et al., 2000). Determining the absorption profile for human melanopsin would determine if the wavelength of light to which melanopsin is most sensitive is associated with effective light therapy for SAD.

Because light is related to the prevalence, seasonal pattern, onset of symptoms, phase-delayed rhythms, and circadian sleep abnormalities in SAD and because of the antidepressant effect of bright light therapy for SAD, investigating the sequence of melanopsin in individuals with SAD may identify a potential biological mechanism. The gene for melanopsin has been identified and sequenced, allowing investigation of its potential function through genetic sequencing. In addition, at least part of the vulnerability to SAD may be the result of heritable factors, making the investigation of the melanopsin gene sequence in SAD a priority.

Genetic Influences on SAD

Studies of families with SAD and similar ethnic groups indicate that SAD runs in families, and twin studies provide some evidence that SAD may be an inherited disorder (Sher et al., 1999). Evidence from twin and family studies for a genetic basis for SAD underscores the need to investigate candidate genes for abnormalities that segregate with SAD, including variations in the gene for melanopsin.

Twin Studies

Twin studies have used self-report measures of seasonality such as the Seasonal Pattern Assessment Questionnaire (SPAQ; Rosenthal, Bradt, & Wehr, 1984). Seasonality is the tendency for seasonal change in eating, sleep, weight, social activity, mood, and

energy level. Using an Australian twin registry, researchers found a significant genetic contribution to seasonal changes in mood and behavior, especially for winter-type seasonality (feel worst in December, January, or February; Madden, Heath, Rosenthal, & Martin, 1996). A seasonality factor identified using multivariate genetic analysis had a heritability coefficient of 29% and accounted for 40% of the phenotypic variance in seasonality symptoms in both male and female twins in the Austrailian registry (Madden et al., 1996). A study of seasonality in twins reared apart compared to twins reared together in Japan indicated that the effect of genetics on seasonality may differ by gender (Jang, Lam, Harris, Vernon, & Livesley, 1998). Specifically, seasonality was significantly heritable among males (accounting for 69% of total variance) and less heritable among females (45% of the variance accounted for; Jang, Lam, Livesley, & Vernon, 1997).

Family Studies

Studies of family members of individuals with SAD in multiple areas (i.e., Canada, Britain, and Switzerland) found prevalence rates for SAD and other mood disorders among first-degree relatives (Lam, Buchanan, & Remick, 1989; Thompson & Isaacs, 1988; Wirz-Justice et al., 1986). Results revealed that 13-17% of relatives had SAD (Lam et al., 1989; Thompson & Isaacs, 1988; Wirz-Justice et al., 1986), compared to 1.4-9.7% SAD in the general population (Rosen et al., 1990). Twenty five to 67% of relatives had nonseasonal mood disorders (Lam et al., 1989; Thompson & Isaacs, 1988; Wirz-Justice et al., 1986), compared to 8-20% of the general population (Blazer, 1995). Research on families in Japan, including spouses, suggests that environmental influences may be more important than genetics, at least among Japanese individuals. One large

Japanese cohort demonstrated no familial association with seasonality of depression symptoms, but a significant correlation between spouses' seasonality, indicating possible social influences on the expression of seasonal patterns of behavior (Sasaki, Sakamoto, Akaho, Nakajima, & Takahashi, 1998).

To date, two twin studies and three family studies support a genetic basis for SAD, whereas one study (Sasaki et al., 1998) found no familial association. Therefore, a preponderance of evidence suggests SAD may have a genetic basis. The question of whether or not SAD has a genetic basis may be resolved by identifying specific genes associated with SAD. This project aims to determine if melanopsin is associated with SAD and may be one gene responsible for a genetic vulnerability to SAD.

Mutations in the Genome and SAD

Because a preponderance of twin and family studies indicate that SAD may be heritable, the search has begun for specific candidate genes responsible for the genetic variance. Two genetic variants related to the neurotransmitter serotonin have been associated with SAD and seasonality (Portas, Bjorvatn, & Ursin, 2000; Wallin & Rissanen, 1994). In all forms of depression, the exact nature of the neurochemical imbalance remains unknown (Wood, Thomas, & Watson, 2002); however, the neurotransmitter serotonin has been indirectly implicated in SAD and nonseasonal depression because of the efficacy of medications targeting serotonergic cells in the brain, particularly the selective serotonin reuptake inhibitors (Jacobs, 2002). The mechanism for serotonin's involvement in depression is not established at present. A serotonin deficiency may be particularly relevant to the SAD symptoms of increased eating, weight gain, hypersomnia, and carbohydrate craving (Wood et al., 2002).

Studies of variations in serotonin-related genes in SAD have contradictory findings. Polymorphisms in serotonin genes are more common among individuals with SAD compared to nondepressed controls (Sher et al., 1999), including 5-HTTLPR and 5-HT_{2A}-1483G/A (Johansson et al., 2003). Recent research on 5-HTTLPR, however, has cast doubt on the association between SAD and this serotonin transporter promoter repeat length polymorphism, finding no association between 5-HTTLPR and SAD or seasonality (Rosenthal et al., 1998). The discrepancy may be explained by a hypothetical, multi-genetic model for SAD in which different genes and factors account for a majority of the variance in SAD in different sub-groups.

A study of twins reared apart found that the total variance in SAD accounted for by genes is 29% - 40% (Sher et al., 1999). Estimates suggests that 5-HTTLPR-S accounts for 5% of the genetic variance in seasonality (Madden et al., 1996), a small portion of the total variance. The genetic influences on SAD most likely result from the combined effects of multiple genes and multiple factors within those genes, including genes not directly related to the serotonin system (Ohguro et al., 2002). Genes mediating non-visual light input pathway or circadian clock represent candidates in the pathogenesis of SAD because of the identified role of melanopsin in rodent non-visual systems, and the role of light in SAD reviewed above. Determining whether or not any variations in melanopsin are associated with SAD in the present study may identify one gene that may be a part of a multi-genetic vulnerability to SAD.

Melanopsin Variant P10L in SAD: Comparison to Functional Changes in Rhodopsin

Rhodopsin is a retinal photoreceptor protein involved in vision (Hwa, Klein-Seetharaman, & Khorana, 2001). Mutations in rhodopsin can lead to Retinitis

Pigmentosa (RP), which is a disease characterized by retinal degeneration. Melanopsin is structurally similar to all opsins, including rhodopsin, because it has a similar extracellular domain and seven transmembrane domains (Provencio, Jiang, De Grip, Hayes, & Rollag, 1998). One particular mutation in rhodopsin, P23H (Liu, Garriga, & Khorana, 1996), is similar to an identified variant in melanopsin (P10L) because it is in the amino terminus of the transmembrane protein, and it is a variant in which a proline is switched to a dissimilar amino acid [i.e., histadine (P23H in rhodopsin) or a leucine (P10L in melanopsin)]. The P23H mutation in rhodopsin is one of many causes of RP (Hwa et al., 2001). P23H is a potential model for how the P10L variant in melanopsin could affect individuals with SAD, but first an association between P10L and SAD must be found.

Study Purpose

The investigation of melanopsin's role in SAD is warranted by several lines of empirical research: melanopsin is a non-visual, circadian photoreceptor; photoperiod is related to SAD onset and severity; light therapy is effective in treating SAD; circadian rhythms are disrupted in SAD; and 29% to 40% of the variance in SAD accounted for by genetic factors. Importantly, polymorphisms in the gene for melanopsin may help to identify a mechanism by which melanopsin may function differently in SAD and either lead to SAD onset, or mediate treatment response in SAD. Etiological hypotheses for SAD suggest there may be an abnormal transmission of environmental light cues through the eyes and, possibly, resultant dysregulation of the circadian clock. Although visual photoreceptors also may be involved in circadian photoreception, both visual photoreceptors and melanopsin are required for normal circadian light entrainment in

rodents. If this finding generalizes to humans, then melanopsin may be involved in human non-visual photoreception. If melanopsin is involved in human non-visual photoreception, then variations in melanopsin may underlie abnormal responses to light observed in SAD. Investigating possible polymorphisms in SAD is the first step to determine whether the function of melanopsin is crucial in SAD.

The purpose of this study is to compare the frequency of a previously identified polymorphism in the melanopsin gene (P10L) in a sample of individuals with SAD (SAD group; $n = 37$) relative to two different groups. The first group is a comparison group that is not ideal for comparison to the SAD sample, but which was available and of substantial size (comparison group; $n = 84$). The second group is a control group comprised of individuals with no history of depression and minimal seasonality with the same gender distribution as the SAD sample, but is a relatively small group (control group; $n = 22$). These data will allow us to determine whether P10L segregates with SAD diagnosis. The central hypothesis was that P10L would be more common in individuals diagnosed with SAD than in both types of controls. If identified, the P10L polymorphism might have clinical utility as a marker for SAD or as a marker of potential treatment response. Using both control groups for separate analyses indicated whether or not future studies are warranted using adequate numbers of minimal seasonality controls with no history of depression. Subsequent to identifying the frequency of any polymorphisms in the gene for melanopsin, the frequency of any identified mutations was used to determine the sample size needed in later studies to achieve sufficient power in statistical analysis.

Methods

Participants

SAD Participants

Community residents, aged 18 or older, were recruited in the Washington, D.C., metropolitan area with print and radio advertisements from October 2000, to October 2003 as part of a randomized clinical trial comparing light therapy, cognitive behavioral therapy, and the combination of these two therapies to treat SAD. Inclusion criteria for the SAD group included a history of Major Depressive Disorder, Recurrent, with Seasonal Pattern, as assessed by the Structured Clinical Interview for DSM-IV Axis I Disorders – Clinician Version (SCID-CV; First, Spitzer, Gibbon, & Williams, 1995). SAD participants also met criteria for a current SAD episode on the Structured Clinical Interview Guide for Hamilton Depression Rating Scale, SAD version during the winter of their recruitment (SIGH-SAD; Williams, Link, Rosenthal, Amira, & Terman, 1992). Out of 83 individuals screened, 22 individuals were excluded from the larger treatment study because they were either engaged in psychotherapy, taking psychotropic medications, or using light therapy; evidenced any additional Axis I disorder including Bipolar-type SAD; had plans for absences or vacation during the winter months of the study; or refused to participate. Sixty-one SAD participants in the larger treatment study were randomized, and 54 completed the entire treatment study, including a 1-year follow up visit, before being given the opportunity to provide a sample for genetic testing. Of the seven participants who did not complete the treatment study, two had medical complications unrelated to the study, one had a medical complication related to light therapy, and four terminated treatment prematurely due to dissatisfaction with group

assignment. Of the 54 treatment study volunteers, 36 (66%) SAD participants consented to participate in this study and provided DNA samples.

Comparison Group

Two sources were initially available to obtain a large number of DNA samples. First, some samples (n = 68) were included from Dr. Yoshitatsu Sei's study on Malignant Hypothermia (MH; USUHS protocol #RO80AA). As part of Dr. Sei's protocol, participants provided DNA samples (i.e., blood). Participants in Dr. Sei's study were given the opportunity to place restrictions on their genetic samples for future studies. The samples provided by Dr. Sei were from participants who chose the option, "My tissue may be used for any scientific purpose involving this or any other project, now or in the future. My tissue may be shared with other researchers. If my tissue is shared with other researchers, they are bound to the limitations set forth in this informed consent agreement." These samples were provided to our research group after being detached from all identifying information including gender and ethnicity. Establishing gender for these delinked samples is unethical given the limitations set forth in the protocol under which the samples were collected. However, summary data on the control sample provided by Dr. Sei indicated that the 68 participants were all Caucasian and 6% were female. This is a significantly different gender distribution than that of our SAD group. The gender difference was subsequently determined to be a significant limitation of the comparison group, leading to the recruitment of a smaller group of individuals with minimal seasonality and no history of depression (i.e., the control group).

Discarded DNA samples provided by the NIH blood bank (n = 16) constituted a second source for a comparison group in this study. These DNA samples were collected by

NIH for use in multiple studies and are provided to outside researchers when no longer necessary to the NIH researchers and after being detached from all identifying information, including gender and ethnicity. Comparison group individuals from both sources were not screened for depression or any psychiatric condition, but are expected to have a frequency of SAD similar to the general population in the Washington, D.C., metropolitan area (6.3%; Rosen et al., 1990), and to have a gender distribution similar to that of the general population in Montgomery County, Maryland (52% female; U.S. Census Bureau). The Institutional Review Board (IRB) at the Uniformed Services University of the Health Sciences (USUHS) approved use of DNA samples from both types of comparison group samples.

Limitations of this comparison group include that 68 had known malignant hypothermia, none were screened for current or past depression, and the gender and ethnicity distribution, although not specifically known, was different from that of the SAD group. Therefore, we sought to recruit an additional group of "true" controls for comparison who were not characterized by a specific physical disease status; were not significantly different from our SAD group on the basis of ethnicity, gender, age, and years of education; had no history of depression; and demonstrated minimal seasonality.

Nondepressed Control Group

Analogous to the SAD group, nondepressed controls, aged 18-years and older, were recruited for a larger psychophathology study through print and radio advertisements in the Washington, D.C., metropolitan area. Recruitment began in December of 2003 and is ongoing. Inclusion criteria for the nondepressed control group included no history of Major Depressive Disorder and no current Axis I disorder, as assessed by the SCID-CV (First et al., 1995). Nondepressed control participants also

scored in the normal range on the Seasonal Pattern Assessment Questionnaire (SPAQ; Rosenthal et al., 1984): (a) a global seasonality score (GSS) of 8 or 9, but reported no problems across the seasons, or (b) a GSS less than or equal to 7. In addition, controls were required to score less than or equal to 8 on the Beck Depression Inventory-Second Edition (BDI-II; Beck et al., 1996), indicating no or very minimal current depression symptoms. Of 41 nondepressed controls in the psychopathology study, 22 consented to the procedures of this study and provided DNA samples during a single laboratory visit.

Procedures

Samples were collected using the Whatman Inc. OmniSwab buccal swab. DNA was extracted from tissue samples using the QIAamp DNA MiniKit following the Buccal Swab Spin Protocol. The QUIamp kit separates DNA from buccal swab tissue through cell lysis, centrifugation, and a microfilter. The DNA is eluted from the filter using deionized water and collected and stored for sequencing. The USUHS IRB approved collection and analysis of DNA from the SAD and nondepressed control samples for this study. DNA obtained from all three participant groups was analyzed spectrophotometrically. Amplification of each of the 10 melanopsin coding regions or exons was completed using polymerase chain reaction (PCR) on an MJ Research DNA Engine Thermal Cycler. The success of the 10 PCR reactions was confirmed by electrophoresis using a 1.5% agarose gel. PCR products were prepared as a sequencing template using Micropure-EZ columns, and Amicon Micron-PCR Centrifugal Filter Devices. Sequencing reactions for each exon used gene-specific forward and/or backward primers and the ABI Verision 2.0 Big Dye Sequencing kit. Sequencing reactions incorporated dye-terminators according to the DNA template (i.e., template-

directed dye-terminator incorporation with fluorescence-polarization detection; FP-TDI), a method designed for detecting single nucleotide polymorphisms like P10L. Sequencing reactions were carried out in the MJ Research DNA Engine using a sequencing program. Reaction products were prepared for sequencing using a Performa DTR Gel Filtration Cartridge (Edge BioSystems) prior to vacuum dessication and re-suspension in Template Suppression Buffer. Finally, reaction products were sequenced on the ABI Prism® 310 Genetic Analyzer using fluorescence-polarization detection. The Basic Local Alignment of Sequences Tool (BLAST; Altschul, Gish, Miller, Myers, & Lipman, 1990) server at the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.gov/BLAST/) allowed confirmation of the identity of each of the 10 exons or coding regions of the gene.

Accurate discrimination between heterozygote and homozygote genotypes requires a reliable sequencing reaction and sequencing instrumentation with high sensitivity to reaction products, a robust signal, and precise control over the electrophoresis parameters that determine the separation of each DNA base allowing easy discrimination between proximal bases. The sequencing chemistry used in this study (ABI Verision 2.0 Big Dye Sequencing kit) is a commercially available template-directed dye-terminator incorporation with fluorescence-polarization detection (FP-TDI; Akula et al., 2002). An independent study of the FP-TDI sequencing reaction found that, given readable sequence output, the discrepancy rate was 0 in 631 samples, and the 95% confidence interval of this proportion ranged from 0.00% to 0.58%, or less than 6 errors in 1000 genotypes (Akula et al., 2002). All data presented below are from readable sequence output, and the complementary strand of 30% of samples was sequenced. Of

the complementary strand sequences, none identified discrepant genotypes. The ABI Prism® 310 Genetic Analyzer has a 98.5% basecalling accuracy under our reaction conditions which is comparable to the accuracy achieved with other commercially available sequencing instruments (Applied Biosystems Publication #237206-005, 2004).

Data Analytic Strategy

The sequence of the melanopsin gene in SAD patients and in both control groups was compared to the published sequence of the human melanopsin gene to identify specific polymorphisms. Each of the ten exons and regions immediately before and after the melanopsin gene was sequenced for each participant. A Pearson's Chi-square analysis was performed to compare the relative frequency of any identified polymorphisms in the melanopsin gene between SAD and control participants. SAD participants were compared to the comparison group and to the nondepressed control group in separate analyses using Pearson's Chi-square. Odds Ratios were calculated to determine if individuals with P10L were more likely to be in the SAD group than in the comparison group or the nondepressed control group.

Results

Participant Characteristics for the SAD and Nondepressed Control Groups

The SAD participants were not significantly different compared to the nondepressed control group on the basis of gender, X^2 (1, N = 59) = 1.34, p = .25, *ns*; ethnicity, X^2 (4, N = 59) = 7.51, p = .11, *ns*; or education level, X^2 (1, N = 59) = 3.52, p = .48, *ns*; (Table 1). In addition, the groups were not significantly different in age, F (1, 59) = 2.16, p = .13, *ns*, with groups predominately middle-aged (SAD M age = 48.6 years, SD = 12.5; nondepressed control M age = 42.17 years, SD = 13.8). Post-hoc power

analysis indicated that the gender, ethnicity, education level, and age analyses were underpowered, but we used the largest combined sample available to test the study hypothesis. In a recent meta-analysis of studies of the association between a serotonin-related gene and suicidal behavior, studies meeting the inclusion criteria had an average sample size of 206, including cases and controls (Lin & Tsai, 2004). Therefore, both the comparison group (N = 120) and control group (N = 58) analyses were performed with samples smaller than needed.

Table 1

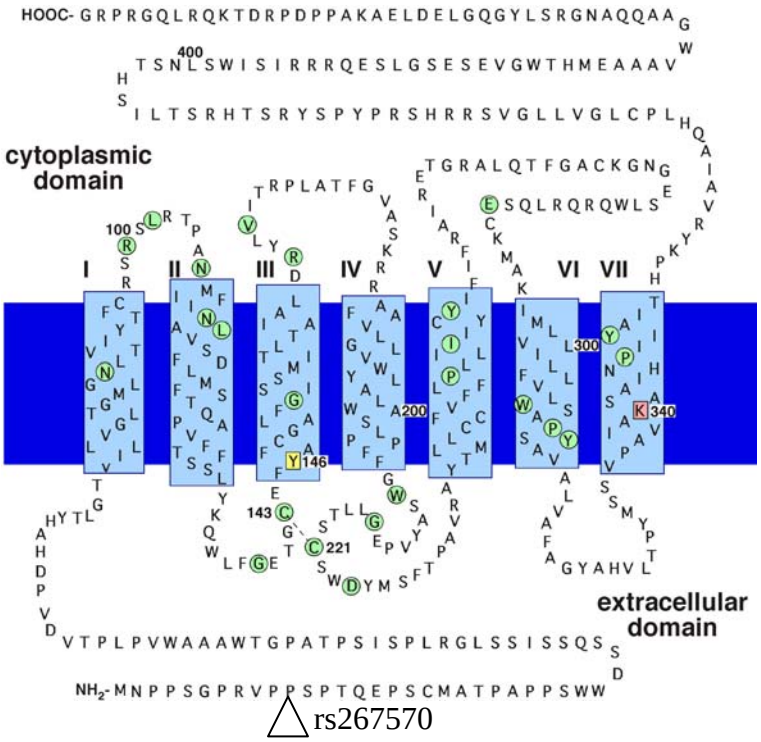
Demographics for the SAD and Nondepressed Control Groups, Number of Participants Followed by Proportion of Each Category in Parentheses

	SAD Group (n = 37)		Nondepressed Control Group (n = 22)	
Gender				
Female	34	(.92)	18	(.82)
Male	3	(.08)	4	(.18)
Ethnicity				
Caucasian	31	(.84)	13	(.57)
African American	3	(.07)	6	(.26)
Hispanic or Latino	1	(.03)	1	(.13)
Asian American	1	(.03)	1	(.04)
Other	1	(.03)	0	(.0)
Education				
14 years or less	7	(.19)	13	(.59)
College Educated & above	30	(.81)	9	(.41)

Description of Identified Variants in the Melanopsin Sequence

A polymorphism in Exon 1 of the melanopsin gene was identified in the SAD sample. This polymorphism (P10L) leads to a change in the protein sequence for melanopsin, so it is referred to as a variation or allelic variant. The variant is a previously reported single nucleotide polymorphism (SNP) on Exon 1, rs2675703, in which the more common C (cytosine) base is replaced with a T (tyrosine). This is a missense mutation, meaning that the resultant amino acid sequence of the protein is changed from a proline to a leucine at amino acid position 10 in the protein (Pro-10-Leu or 'P10L'). Amino acid position 10 is part of the extracellular amino terminus (white arrow in Figure 1; Provencio et al., 1998). In Figure 1, the seven transmembrane domains of the protein are depicted in the cell membrane, with the portion prior to domain I being part of the extracellular amino terminus, and the portion subsequent to domain VII being part of the cytoplasmic domain (Provencio et al., 1998). The proline in the 10^{th} position is the amino acid that varies in P10L.

Figure 1. Human melanopsin structure.



Source: Provencio et al. (1998).

Proline, the standard amino acid at position 10, is a cyclic aliphatic amino acid that does not favor formation of alpha-helices, but rather causes the three dimensional structure of a protein to have a kink or break in the alpha-helix section of a protein (Karvonen et al., 1998). A change in the three dimensional structure of melanopsin by the P10L variation may lead to a functional change in the resultant protein, making the identification of P10L in our SAD sample a meaningful discovery. In the extracellular amino terminus, it is expected that the proline causes a sharp bend in the 3-dimensional structure in the normal variation, but this is not expected when the leucine variant is present. Leucine has a hydrophobic side chain and favors formation of alpha-helices.

Hydrophobic amino acids are more commonly found in the transmembrane domain of a protein that is hydrophobic.

Frequency Analysis of P10L

The P10L variant was identified in the SAD group and in both control groups at the frequencies reported in Table 2. Frequencies were calculated for each allele, for each genotype combination if present in the sample, and for the alternate forms (i.e., C/T and T/T) together to compare with the more common, wild-type genotype (i.e., C/C). Because individuals have two alleles for every locus, an individual can have two copies of the more common allele (C/C), two copies of the alternate allele (T/T), or one of each (C/T). Individuals with the C/C genotype will have normal amounts of the normal protein sequence transcribed. Individuals with the T/T genotype will have two copies of melanopsin coding for the alternate form of the protein. Individuals with C/T will have one DNA sequence that codes for the normal protein, and one coding for the variant, so these individuals have intermediate amounts of the two protein sequences.

Table 2

Frequency of Genotype and Alleles in the P10L Variation in Melanopsin, Number of Participants Followed by Proportion of Group in Parentheses

	Genotype Frequency				Allele frequency	
	C/C	C/T	T/T	C/T & T/T	C	T
SAD Group (n = 36)	26 (.72)	10 (.28)	0 (.0)	10 (.28)	62 (.86)	10 (.14)
Comparison Group (n = 84)	71 (.85)	12 (.14)	1 (.01)	13 (.15)	154 (.92)	14 (.08)
Nondepressed Control Group (n = 22)	18 (.82)	4 (.18)	0 (.0)	4 (.18)	40 (.91)	4 (.09)

SAD vs. Comparison Group on P10L Frequency

No comparisons between the SAD and comparison groups were statistically different although the P10L variant was descriptively more common in the SAD group (28%) than in the comparison group (15%; Table 2). The SAD participants were not statistically more likely to have one or more variations (i.e., C/T or T/T) compared to the comparison group, X^2 (1, N = 120) = 2.46, p = .12, *ns*. The power of this overall analysis was calculated using *w*, an effect size index for Chi-Square tests, and Pearson's coefficient of contingency (*C*), a measure of association for contingency tables (Cohen, 1988). The index of effect size in this Chi-Square (w = .168) was small by Cohen's standards (small = .1, medium = .3, large = .5; Cohen, 1988), and power was determined to be between 19% and 59% for this analysis. An N of between 200 and 800 participants is required to achieve the desired standard of 80% power in a subsequent analysis with this effect size (Table 7.3.15, page 235; Cohen, 1988).

The overall Chi-square was followed with post-hoc tests to compare the frequency of all three genotypes among groups (i.e., C/C, C/T, and T/T) as well as allelic frequency between groups (i.e., C's and T's). When all three genotypes were compared, results indicated no significant difference among groups, X^2 (2, N = 120) = 3.40, p = .18, *ns*. When allelic frequency was compared between groups, no significant difference in frequency of the variant in the SAD participants was found, X^2 (1, N = 240) = 1.73, p = .19, *ns*. Individuals with P10L were not significantly more likely to be in the SAD group than in the comparison group, OR = .48, 95% CI = .19–1.22. The genotype and allelic frequency analyses had effect sizes that were small or less than small (w = .143 and .085,

respectively) and both analyses were insufficiently powered (19-59% and less than 19%, respectively; Cohen, 1988).

SAD vs. Nondepressed Control Group on P10L Frequency

No comparisons between the SAD and the nondepressed control groups were statistically different, although the P10L variant was descriptively more common in the SAD group (28%) compared to the nondepressed control group (18%; see Table 2). The SAD participants were not statistically more likely to have one or more variations (i.e., C/T or T/T) compared to the nondepressed control participants, X^2 (1, N = 58) = .687, p = .41, *ns*. Power for this analysis was determined to be 12%, which is substantially below the standard of 80% power (Table 7.3.15, page 235; Cohen, 1988). The calculated effect size was w = 0.109 which is small by Cohen's standards (page 224; Cohen, 1988), and would require a sample size of 800 to achieve 80% power (Cohen, 1988).

The overall Chi-square was followed with post-hoc tests to compare allelic frequency between groups (i.e., C's and T's). When allelic frequency was compared between the SAD and nondepressed control groups, the groups did not differ in frequency of the variant, X^2 (1, N = 116) = .59, p = .44, *ns*. Individuals with P10L were not significantly more likely to be in the SAD group than in the nondepressed control group, OR = .58, 95% CI = .16–2.13.

Discussion

This is the first investigation of polymorphisms in the gene for melanopsin in SAD. A sample of 37 individuals diagnosed with Major Depression, Recurrent with Seasonal Pattern was compared to the comparison group and to controls with no history of depression and minimal seasonality. The comparison sample was not matched to the

SAD sample on the basis of gender and ethnicity and was not screened for depression. Also, 68 individuals in the comparison group had known malignant hypothermia (MH), and 16 samples were of unknown health status. Although we did not expect MH status to be differentially related to SAD prevalence relative to the prevalence of SAD in the general population, the potential confound of this factor and gender cannot be disputed. The SAD group was 92% female, whereas the individuals with MH in the comparison group (n = 68) were 6% female and we expect the samples from NIH (n = 16) to be 52% female, such that the comparison group on the whole is likely to be 15% female. Allelic frequency does vary across ethnic groups and gender for many genetically-based conditions. Therefore, a comparison between individuals with SAD and gender- and ethnicity-matched control participants with no history of depression and low seasonality scores was undertaken (nondepressed control group), but only smaller than desired sample size (n = 22) was available. The limitations of this study include small sample size, the lack of specific demographic information for the comparison group, a greater proportion of men in the comparison group, and a high prevalence of known malignant hypothermia in the comparison group.

No statistically significant differences were discovered in analyses comparing the SAD group to either control group on P10L frequency. The P10L variant was found in 28% of the individuals with SAD in this study. The P10L variant was identified in 15% of individuals in our comparison group sample and 18% of individuals in the nondepressed control group. Descriptively, the different percentages of P10L in each group suggest that there may be a greater association between SAD and the P10L variant relative to controls in a sufficiently powered test. A suggested association between SAD

and P10L is extremely tenuous given that no tests of the association were statistically significant. In addition, the effect size related to the comparative frequency in P10L between SAD and control participants was small and suggested that 200-800 participants would be needed to achieve 80% power.

Clinical Implications

An attenuated response to environmental light would be relevant to both the photon-count and phase-shift hypotheses described above. Because of melanopsin's role in circadian photoentrainment, variations in the sequence of melanopsin may lead to a less functional circadian photoreceptive protein. As functional variation, P10L, in theory, could make individuals with this variant either more or less sensitive to light. A variation in melanopsin could have three implications for SAD. First, having P10L could make individuals more likely to express SAD. The results of this study do not support this hypothesis. Second, having P10L could make individuals more or less responsive to light therapy for SAD. Third, an additive or interactive combination of these effects may manifest such that individuals with P10L are both more likely to have SAD, and differentially responsive to light therapy as compared to individuals without P10L. Meta-analytic findings suggest that 47% of SAD patients do not fully remit with light therapy (Terman et al., 1989). Therefore, future work in this area should seek to answer a question with substantial clinical utility: Is the P10L genotype predictive of responsiveness to light therapy in SAD? Findings regarding treatment response on the basis of genotype would provide clinicians with some guidance in selecting optimal treatment options for specific SAD patients.

Research to clarify the role of melanopsin in retinal mechanisms of human photoentrainment would facilitate optimization of the light therapy dose to phase-shift circadian rhythms in SAD. Parameters that require elucidation include the intensity, duration, timing, and particular wavelength of light that produces the greatest antidepressant response. If melanopsin-containing photoreceptors mediate the antidepressant response to light in SAD, wavelengths of light that maximally stimulate melanopsin could be emphasized. However, if, in the absence of functional melanopsin-containing photoreceptors, rod photoreceptors are mediating the antidepressant response to light, wavelengths of light known to maximally stimulate rods could be emphasized.

It may be difficult to conclusively demonstrate a link between polymorphisms and SAD for multiple reasons. SAD is likely to be a polygenic and polyfactorial disorder involving not only genetic but also environmental, cultural, psychological, and/or social risk factors. Multiple genes, and multiple variations within genes, are likely involved in SAD as opposed to a single polymorphism in the melanopsin gene alone. There are other molecules in the retina, RHT, and SCN that play a part in circadian rhythms and are potentially involved in SAD. Genes for these molecules (i.e., h*Per*2) are currently being evaluated in other disorders with a known circadian component. When polymorphisms are found, the next step is to determine whether or not a particular polymorphism results in changes in the amino acid sequence of the protein, leading to an unstable protein. This is the case with P10L as well as a mutation in rhodopsin that leads to retinal degeneration (Sher, 2001). Proper functioning of melanopsin is but one step in the chemical cascade within photoreceptive cells in the retina, leaving the possibility that other molecules in

RGCs or in downstream circadian signaling processes constitute a biological mechanism in SAD.

Future Directions

Sufficiently powered tests of the association between P10L and SAD, research on the heritability of the P10L variant and its segregation with SAD symptom presentation, as well as studies of the functional significance of the P10L variation, are needed to determine the inheritance pattern and functional impact of P10L or other melanopsin variants. First, a larger sample of nondepressed controls and a larger sample of SAD participants is needed to achieve sufficient power to determine whether there are significant differences between the groups in the relative frequency of P10L. Therefore, our next step is to utilize the proportions obtained in this study to design an adequately powered comparison between individuals with SAD and a group of gender- and ethnicity-matched controls with no history of depression, no current Axis I disorder, and minimal seasonality. To establish heritability, first-degree family members of individuals with SAD who have the P10L variant, including parents, siblings, and children could be assessed to determine if the P10L variant segregates with SAD diagnosis among relatives. To this end, we could determine whether or not P10L segregates with SAD in families. It will be important to determine whether P10L segregates with SAD diagnosis, treatment response, or both in certain families but not others, suggesting different risk factors for SAD in different families.

Genetic Counseling

Individuals with a genetic predisposition to disease often seek counseling to improve understanding of their own prognosis and to inform their reproductive decisions.

Genetic counselors can help identify and interpret the risks of an inherited disorder, explain inheritance patterns, and explain the meaning of the medical science involved. Counselors also can provide support and address any emotional issues raised by the test results. Opportunities exist for genetic counseling and risk assessment for a variety of psychiatric conditions including depression. Counseling includes constructing a family history, discussion of the risks each family member faces, and education about the multiple causes of disease. Currently, a link between P10L and SAD is not evident. However, referrals to genetic counseling centers may be warranted if individuals involved in further research on genes in SAD request information about genetics and depression, in general, and familial transmission of genetic risks.

Conclusions

Pathophysiological theories of SAD propose an abnormal response to low light levels in the winter. Hypotheses invoke biological mechanisms for physiological dysregulation and resultant depression during the winter, but have not specified proposed molecular mechanisms underlying the physiological change. Given melanopsin's role in transmitting light information to non-visual centers including those involved in circadian photoentrainment, melanopsin is a good candidate for a molecular mechanism underlying the pathology of SAD. These preliminary data indicate that, in this sample, a potentially functional variation in the melanopsin sequence is not statistically more prevalent in individuals with SAD compared to the comparison group and nondepressed controls. Although this does not suggest a role for melanopsin in SAD, further work may investigate that possibility. Primarily, a comparison utilizing a large ($N > 200$) gender- and ethnicity-matched control group screened for depression and other Axis I disorders

may be sufficiently powered to detect an association. Subsequently, studies of family members of individuals with the P10L variant may help to establish segregation of the P10L variant with SAD in a heritable pattern in some families. Studies of potential three-dimensional change in the melanopsin protein resulting from P10L may identify the molecular functional significance of P10L in the retina. Future work should also examine whether melanopsin variants may mediate light therapy's antidepressant effects in SAD to inform treatment recommendations.

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Footnotes

[i] Living organisms with endogenous biological timing systems include, but are not limited to: monarch butterflies, sea turtles, whales, migrant birds, insects, and mammals such as wildebeest, caribou, and reindeer, numerous species of migrant fish, sea mammals, and penguins, lizards, crabs, squirrels, chipmunks, marmots, jumping mice, and some bears.